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FEATURE ARTICLE

The BacVector System: Simplified Cloning and Protein Expression Using Novel Baculovirus Vectors

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The baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV) has become a popular vehicle for the cloning and expression of recombinant proteins in insect cells (1, 2). The baculovirus expression vector system (BEVS) offers significant advantages over prokaryotic and other eukaryotic systems for the production of many proteins (see sidebar on p. 2). To complement the pET System for protein expression in bacteria, Novagen now offers the BacVector™ System, a novel and complete set of reagents and kits for expression in insect cells. The BacVector System provides a number of key improvements over other baculovirus systems, including:

- **BacVector Transfection Kits**, containing **BacVector-1000 or BacVector-2000 Triple Cut Virus DNA and Eufectin™** Transfection Reagent; BacVector-2000 has been deleted for several non-essential genes that compete with target protein production,
- **pBAC™ *E. coli* LIC Kits and Transfer Plasmids,** for convenient cloning and high-level expression for various applications, including fusion tags for detection and purification, optional *gus* marker gene for positive identification of recombinants, and co-expression of multiple genes. *E. coli* LIC (ligation-independent cloning) kits contain prepared LIC transfer plasmid,

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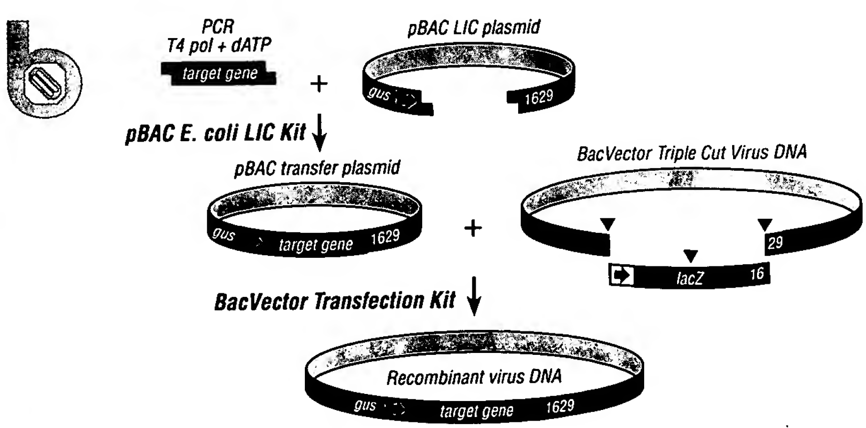

Fig. 1. Construction of baculovirus recombinants with the BacVector System
Target genes are cloned into pBAC transfer plasmids, isolated from *E. coli*, and co-transfected with BacVector Triple Cut Virus DNA into insect cells to produce recombinant baculovirus vectors. This diagram shows the strategy of ligation-independent cloning (LIC) for the *E. coli* cloning step, which is available with pBAC-2cp and pBACgus-2cp transfer plasmids. See text for further details.

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Pellet Paint: A Visible Nucleic Acid Carrier for Efficient, Error-Free Precipitation

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Most procedures in molecular biology require the recovery of small amounts of nucleic acids by precipitation in the presence of salt and alcohol. Unfortunately, commonly used microgram quantities of DNA and RNA are often difficult to see as pellets following centrifugation. In addition, many protocols entail incubations at low temperatures and/or high-speed centrifugation for prolonged periods in order to increase the recovery of dilute molecules. Valuable samples are often lost due to incomplete precipitation or difficulty in visualizing the pellet, which is usually very tiny, clear or translucent, sometimes dispersed along the tube walls, and which can become easily dislodged and inadvertently discarded during washing steps. The use of co-precipitant "carriers" such as glycogen or tRNA can improve recoveries, but many researchers are reluctant to use these reagents due to incompatibility with downstream procedures or uncertainties about purity.

To solve these problems, we have developed Pellet Paint™ co-precipitant*, which is a brightly colored polymeric carrier molecule designed specifically for nucleic acid precipitation. Pellet Paint allows precipitated material to be seen as a vivid pink pellet under normal lighting conditions, and as a fluorescent pellet under UV light (Fig. 1). This reagent greatly improves the efficiency, speed and reliability of virtually any procedure requiring precipitation of small amounts of nucleic acids. Even minute amounts (less than 2ng/ml) of nucleic acids are recovered using the standard 5 minute protocol. The Pellet Paint glycogen-based polymer is chemically inert and has been tested for compatibility with a variety of applications. Furthermore, since the preparation is free of endogenous nucleic acids, it can be used with confidence that no impurities are introduced to the sample. A comparison of the properties of several carriers is shown in Table 1.

Another advantage of Pellet Paint is that it forms tight pellets which do not spread along the tube walls. Thus, the exact location of the pellet is always known.

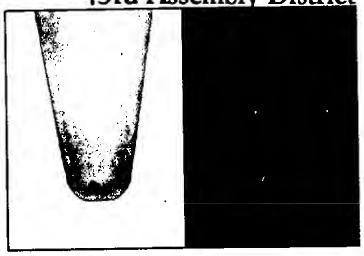

Fig. 1. Pellet Paint pellet (2µl) under visible and UV illumination

Solubilization of the pellet is also easily followed as the Pellet Paint dissolves along with the co-precipitated sample.

We have tested the effect of Pellet Paint on the subsequent use of DNA or RNA for many applications, including those listed in Table 1. In addition, we have studied the recovery of molecules of various sizes. Some of our data are presented below.

Rapid, Efficient Precipitation

Pellet Paint is compatible with the use of sodium acetate, sodium chloride and ammonium acetate salts. In the standard protocol, which applies to both DNA and RNA, 2µl of Pellet Paint is added to the sample, followed by 0.1 volume sodium acetate and two volumes of ethanol. When volumes are limiting, 1 volume of isopropanol may be used instead of ethanol. After mixing, the sample is immediately centrifuged at 12,000 × g for 5 minutes. Pellets can be rinsed with ethanol and dried prior to resuspension.

	Pellet Paint	glycogen	tRNA
easily visualized	✔	–	–
free of DNA and RNA	✔	?	–
compatible with:			
gel electrophoresis	✔	✔	–
PCR amplification	✔	?	–
DNA sequencing	✔	✔	–
restriction digestion	✔	✔	✔
ligation	✔	✔	?
transformation	✔	?	–
cDNA synthesis	✔	?	–
kinase reactions	✔	✔	–
random priming	✔	?	–
in vitro transcription	✔	✔	?
in vitro translation	✔	✔	✔

Table 1. Comparison of different carriers for precipitation of nucleic acids

To determine the size range of products recovered under standard conditions, we precipitated a ^{32}P-labeled sequencing reaction with Pellet Paint and analyzed the recovered material on a sequencing gel (data not shown). Overexposure of the gel showed that DNA molecules larger than 68 bases were recovered efficiently, while faint bands were observed from 68 to 50 bases, and virtually no signal was observed below 50 bases. These results indicate that small single and double stranded molecules and unincorporated mononucleotides are efficiently removed using the Pellet Paint method.

In other experiments, dilutions of ^{32}P-labeled RNA and duplex DNA synthesized *in vitro* by transcription and random priming, respectively, were precipitated in the presence of Pellet Paint. The data in Table 2

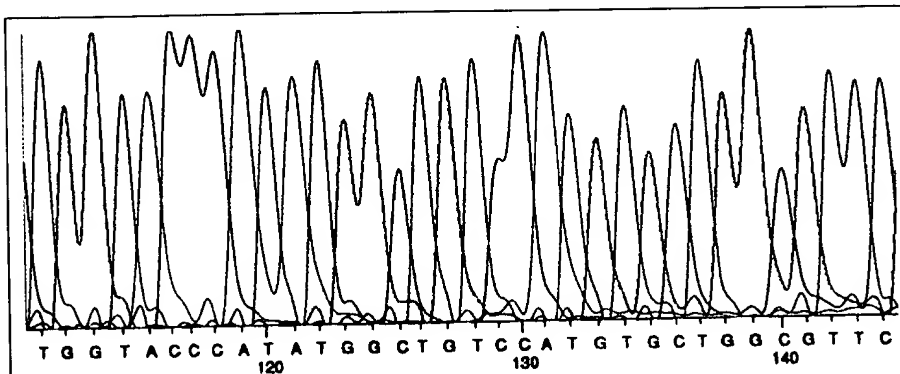

Fig. 2. Automated sequencing with Pellet Paint

A double-stranded plasmid template (5µg) was precipitated with Pellet Paint, denatured with alkali, neutralized and sequenced with a Cy5-labeled primer on a Pharmacia ALF Express sequencer. The sequence was readable to more than 500 bases, which was the same as a control reaction performed in the absence of Pellet Paint. (Data courtesy of M. Domanico, Pel-Freez.)

* patent pending

Sample	incorp. cpm recovered
RNA (100nt, 0.2ng/µl)	90%
RNA (1000nt, 0.2ng/µl)	92%
RNA (10,000nt, 0.2ng/µl)	89%
DNA (100-2000bp, 4pg/µl)	86%

Table 2. Recovery of various RNA and DNA samples with Pellet Paint as the carrier

The indicated samples of ^{32}P-labeled RNA and DNA were prepared using standard protocols for *in vitro* transcription and random priming, respectively. Following the labeling reactions, incorporation was determined by DE81 filtration. Known amounts of incorporated material (300,000 cpm) were precipitated in the presence of Pellet Paint. Samples without Pellet Paint resulted in a 5-50-fold reduction in recovery.

indicate that excellent recoveries of very dilute molecules were obtained using Pellet Paint; furthermore, unincorporated nucleotides were removed as effectively as when DE81 filtration was used. Gel analysis (not shown) verified the absence of unincorporated nucleotides in the precipitated material.

Sequencing

Many DNA sequencing protocols, such as those using double stranded templates, require the precipitation of small amounts of DNA before primer annealing. The loss of the pelleted template is a frequent cause of sequencing reaction failure and can be avoided by the incorporation of Pellet Paint into the precipitation step. Fig. 2 shows Pellet Paint had no effect on the sequence data obtained from a Pharmacia A.L.F. Express automated sequencer using Cy5 primers. Similarly, no effect was seen on sequencing using a radioactive, manual method (data not shown).

PCR

Pellet Paint is especially useful for the precipitation of small amounts of genomic DNA in preparation for PCR analysis. The speed and reliability of the precipitation step afforded by Pellet Paint improves the ability to process multiple samples and increases the confidence of interpreting negative reactions. Fig. 3 shows the results of a highly stringent PCR application in which allele-specific primers were used to amplify genomic DNA precipitated with Pellet Paint. The quality and recovery of the sample DNA is vital to this method, in which polymorphisms are identified based on the presence or absence of an amplified product arising from single base matches or mismatches at the 3' end of a primer. Clearly, the presence of Pellet Paint in the DNA allowed accurate discrimination and amplification from the allele-specific primers.

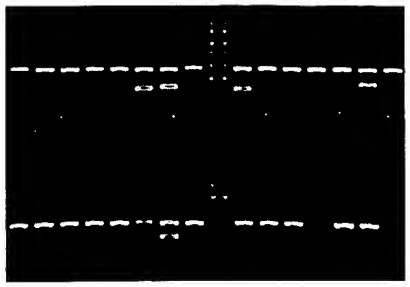

Fig. 3. Allele-specific PCR amplification of genomic DNA precipitated with Pellet Paint

Genomic DNA was isolated from human whole blood using a modified salting out procedure, with 1µl Pellet Paint added before the final ethanol precipitation. The DNA was resuspended in water and used in parallel amplification reactions with sequence-specific primers corresponding to various alleles within the HLA DR and DQ regions (Perkin-Elmer reagents and Model 9600 thermal cycler). An internal control primer yielding a 429bp product was present in each reaction. Allele-specific amplification is indicated by the presence of smaller products in addition to the control band. (Data courtesy of S. Koller, Pel Freez.)

Transformation

Since *E. coli* transformation appears to be sensitive to a variety of compounds and often follows a number of precipitation steps in which Pellet Paint can be used, we tested its effect on transformation efficiency. Plasmid DNA samples (25ng) precipitated with Pellet Paint, glycogen and no carrier molecule were transformed into NovaBlue competent cells. Pellet Paint and glycogen resulted in a four-fold greater yield of colonies (2×10^7 colonies/µg) when compared to the control sample without carrier (4.7×10^6 colonies/µg).

cDNA Synthesis

The preparation of cDNA libraries requires that small nucleic acid samples be precipitated at various points throughout the procedure. Starting with mRNA, some cDNA synthesis protocols may require as many as three or four precipitation steps prior to vector ligation. Inefficient precipitation steps or loss of pelleted material result in a cumulative reduction in the number of primary recombinants. Our tests have shown that Pellet Paint has no effect on first or second strand cDNA synthesis. Since there is also no effect on ligation, restriction digestion, lambda packaging, or transformation, Pellet Paint can be used to ensure efficient recovery of cDNA following linker ligation and size fractionation steps.

Unlike tRNA, which has been traced as the source of non-target sequences in cDNA libraries, Pellet Paint does not contribute contaminating nucleic acids during library construction. The added benefit of pellet visibility allows valuable cDNA reaction products to be protected throughout the procedure.

Limitations

One limitation of Pellet Paint we have found is that the fluorophore absorbs in the UV range, therefore preventing the spectrophotometric measurement of DNA and RNA concentrations. We, therefore, do not recommend the routine use of Pellet Paint during procedures that require spectrophotometric measurements. However, such procedures usually involve higher concentrations (> 100µg/ml) of DNA and RNA, which are more easily precipitated and visualized in the absence of carriers.

In addition, the fluorophore in Pellet Paint interferes with the rhodamine-labeled primers and terminators used by Applied Biosystems automated sequencers, and therefore, may also interfere with other methods based on rhodamine detection. Additional visible co-precipitants for applications that require spectral measurements or rhodamine as a reporter are being developed.

Summary

We have developed a novel reagent for routine use that improves the recovery of small amounts of nucleic acids and speeds the process of precipitation. The high visibility and ease of use of Pellet Paint co-precipitant simplifies nucleic acid manipulation and reduces experimental failures due to losses of critical samples.